1.0 INTRODUCTION

1.1 Background

Lung cancer remains one of the major health problems in the Western world, despite a myriad of new cytotoxic drugs which have been introduced over the last two decades. Eighty-five to ninety percent of patients who develop lung cancer will die of the disease. Clearly, new paradigms based upon advances in understanding the biology of lung cancer need to be entertained in the treatment of this disease.

Symptoms related to uncontrolled intrathoracic spread of tumor is a major problem for many patients with lung cancer. It is estimated that as many as 75% of patients will experience hemoptysis, shortness of breath or post-obstructive pneumonia secondary to endobronchial disease, and that 30% of patients will experience pain due to chest-wall involvement. Radiotherapy is commonly administered to palliate these symptoms, at lower doses than are usually given for patients who present with locally advanced disease and are treated with "curative" intent. Although palliative doses of radiotherapy will often be effective in temporarily improving symptoms, approximately 50% of patients will develop progressive disease.

Alterations of the p53 gene or protein are one of the most common mutations identified in human cancers, and appear critical in the pathogenesis and progression of many tumors. In nonsmall cell lung cancers (NSCLC), mutations are found in approximately half of all tumors studied.

1.2 p53 Tumor Suppressor Gene

The p53 gene is a tumor suppressor gene located on chromosome 17p, which controls cell proliferation and suppresses neoplastic transformation. The wild type p53 protein acts as a cell-cycle regulator at the G1/S interphase which facilitates DNA repair and prevents the propagation of mutations and chromosomal rearrangements to the next cell generations (1, 2). In addition, it also directs cells into the apoptotic cascade, especially if DNA damage cannot be fixed. Thus in order for cancer cells to survive, they often mutate p53 and may become resistant to this sort of apoptotic cell death (3). One copy of the chromosomal region, 17p13, which contains p53, is frequently deleted in both SCLC and NSCLC, and mutational inactivation of the remaining allele occurs in more than 90% of SCLC and 50% of NSCLC (4-7). Furthermore, reintroducing a wild-type p53 gene into lung cancer cells in experimental situations dramatically inhibits tumor cell growth and promotes tumor cell death despite the presence of mutations in multiple other genes (9). It is postulated that since p53 mutant tumor cells survive radiation damage by not undergoing apoptosis, reintroduction of wild-type p53 may, therefore, represent a potential radiosensitizing strategy. The object of this proposal is to take advantage of these properties of p53 and the biology of lung cancer to test the feasibility of the therapeutic reintroduction of p53 via recombinant adenovirus in a multi-institutional setting.

1.3 Adenoviral-Mediated Wild-Type p53 as Radiation Sensitizer

One of the functions of wild-type p53 is to modulate programmed cell death by DNA-damaging chemotherapeutic drugs or ionizing radiation; increased resistance to either chemotherapy or radiotherapy occurs if the wild-type is mutated (10-13), and programmed cell death cannot be executed.

Wild-type p53 gene transfer into the SW620 colorectal carcinoma cell line was performed using the replication-defective adenovirus, Ad-p53, to evaluate the effect of wild-type p53 expression on radiation sensitivity (13). The results indicated that infection with Ad-p53 sensitized the cells. The survival at 2 Gy was reduced from 55% to 23%. Flow cytometric analysis of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling

(TUNEL) assay-labeled cells and *in situ* TUNEL staining of xenograft tumors demonstrated an increase in labeled cells with combination treatment, indicating increased apoptosis in cells treated with Ad-p53 before irradiation. A significant enhancement of tumor growth suppression by this combination strategy was observed in a subcutaneous tumor mouse model compared to p53 gene therapy alone. The delay in regrowth to control tumor size of 1000 mm^3 was 2 days for 5 Gy, 15 days for Ad5/CMV/p53, and 37 days for Ad5/CMV/p53 + 5 Gy, indicating synergistic interactions. These data indicate that the delivery of wild-type p53 to cells with p53 mutations increases their radiation sensitivity, and this may be accomplished by adenoviral-mediated gene therapy.

1.4 Ad-p53

Ad-p53 is a nonreplicating adenoviral vector which encodes a wild-type p53 gene driven by the CMV promoter. The Ad-p53 backbone is an E1-partial E3-deleted human adenovirus type 5 serotype. E1 and E3 gene products modulate viral replication and host immune responses. Adenoviruses are double-stranded DNA viruses with a known tropism for aerodigestive epithelium and are linked to only transient, minor respiratory disease in humans (8). These viruses enter cells via receptor-mediated endocytosis. Although they migrate to the cell nuclei, where they express their genes, they remain extrachromosomal and do not integrate into the host genome. The transient nature of gene expression and the lack of a significant potential for insertional mutagenesis after adenovirus gene delivery allow selective molecular intervention with a low risk of stable integration of the recombinant vector into nonmalignant cells.

Transduction of many different therapeutic genes using recombinant adenoviruses has led to significant antitumor effect in several animal models. Preclinical *in vitro* and *in vivo* murine studies in head and neck cancer and nonsmall cell lung cancer demonstrated a significant antitumor effect of Ad-p53. Initial experiments in which the H358 (a p53-null BAC cell line), H322 (p53 mutant), and H460 (p53 wild-type) human nonsmall cell lung cancer cell lines were treated with Ad-p53 resulted in significant inhibition of cell growth in the H358 and H322 lines (12).

The Ad-p53 gene has been administered to advanced NSCLC patients by intratumoral injection (14, 15). Patients were treated with or without 80 mg/m^2 of cisplatin 3 days before monthly administration of Ad-p53 by either endobronchial or percutaneous injection. No toxicities attributable to either the wild-type p53 gene or the adenovirus vector were observed with up to 6 monthly injections. Two patients had tumor regression with 10^8 plaque-forming units (pfu) of Ad-p53 plus cisplatin, including a patient who failed cisplatin. Adenoviral DNA was documented in 10 of 12 evaluable specimens by polymerase chain reaction (PCR); postinjection tumor biopsies revealed p53 transgene expression by reverse transcriptase PCR (RT-PCR) of immunohistochemistry in 6 of 8 evaluable specimens. Serial biopsies showed increased tumor necrosis and apoptosis by *in situ* TUNEL staining. Neutralizing antibodies were also seen with repeated Ad-p53 injections.

Toxicity of wild-type p53 to normal cells has not been observed, including after direct injection into grossly normal tissues surrounding the site of resection of head and neck tumors. Containment issues are a potential concern, but careful analyses of hundreds of clinical samples both in the US and France have shown no transmission of endobronchially administered Ad-p53 to staff, and the studies are currently being done on an outpatient basis (RPR/Gencell meeting, Miami, 6/97).